A QUICK TEST FOR ESTIMATING BACTERIAL COUNT IN RAW MAPLE SAP

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A QUICK TEST FOR ESTIMATING BACTERIAL COUNT IN RAW MAPLE SAP

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ABSTRACT

This publication describes a modified resazurin dye reduction test that will enable maple sirup producers to estimate rapidly the bacterial count in raw maple sap. Sap of poor sanitary quality which would yield an inferior maple sirup can be identified in less than 2 hours' total time for the test.

INTRODUCTION

Maple sirup producers have long known that raw maple sap is a highly perishable material. The old maple axiom that "first run sap makes the best sirup" and the equally old belief that "a good maple man never lets a drop of sap see two sunsets" are based on practical experience. Colonial maple producers not aware of the existence of microbes were well aware that "old" sap made low quality sirup. Therefore, they tried to apply the one rudimentary sanitation technique available to them--the rapid processing of sap to sirup, in an effort to produce as much sirup of good flavor and color quality as possible. This was obviously a vain effort. As the maple season progressed, warmer weather brought about the rapid growth of micro-organisms, and much of a year's sirup production would be of dark amber or "commercial" color grade.

Since 1950, maple producers, following the lead of the dairy industry, adopted good sanitation procedures which have enabled them to produce a greater percentage of their crop as light or medium amber sirup. Germicidal taphole pellets, ultraviolet lights, and new equipment sanitizing compounds have contributed much to this advance in maple technology. The maple producer, however, has little control over the sanitary quality of sap before it is delivered to his evaporator plant. The sap of low sanitary quality is often boiled down to sirup. The resulting sour, musty, or ropy sirups are products of questionable marketability. Consumer reaction to products of this type rules out repeat sales.

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The effect of microbial contaminants in raw sap on the quality of maple sirup produced from the sap has been well documented.[2/] After sap is received at the evaporator plant, further microbial degradation of the sap can be minimized, but it would be advantageous for the evaporator operator to have a simple method for rapid identification of sap of low sanitary quality after it has been delivered to him. Thus, he could segregate sap which might produce a low grade sirup. In extreme cases, it would enable him to discard sap that would yield an unmarketable sirup. For this purpose, a test was developed at Eastern Regional Research Center by modifying the standard resazurin dye reduction method used in the dairy industry[3/] for estimating the bacterial population of raw milk. The method is given below.

MATERIALS

Resazurin dye.-- Standard resazurin dye tablets certified by the Biological Stain Commission are available in bottles of 100 tablets from Allied Chemical, P.O. Box 431, Morristown, N.J. 07960.[4/] Place 200 ml of distilled water in amber glass bottle and sterilize the bottle in a pressure cooker at 15 pounds pressure for 15 minutes. Remove and with dry sterile forceps add 1 standard resazurin dye tablet. Shake the bottle to insure complete solution of dye before water cools. Store in cool, dark place. Prepare fresh solution weekly.

Nonfat milk solution.-- Dissolve 100 g instant nonfat dry milk in 500 ml distilled water. Sterilize in a pressure cooker at 15 pounds pressure for 15 minutes. Milk should be light tan in color--do not char it. Charred milk imparts an "off" color to the test solution.

Color standards.-- Munsell standard color swatches 7.5 PB6/10 (purplish purple blue) and 2.5 P6/8 (bluish purple) are available from Munsell Color Co., Inc., 2441 N. Calvert St., Baltimore, Md. 21218.

Incubator or water bath.-- An oven with good temperature control or a blacked-out aquarium with a tropical fish tank heater serves as an incubator. Even a box fitted with a small heater and a bimetallic strip thermostat can serve as an incubator. Temperature must be controlled at 98.6° ± 1° F.

2/ Sipple, L., Kissinger, J. C., and Willits, C. O. Ultraviolet irradiation techniques to preserve maple sap. U.S. Dept. Agr., Agr. Res. Serv. 73-64, 19 pp. 1970.

3/ Walter, W. C. (ed). Standard methods for the examination of dairy products. 12th ed. 1967. American Public Health Assoc., New York.

4/ Mention of a company or trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned. This is given solely for the purpose of providing specific information.

Serological pipets.-- Sterile, plastic throw-away pipets, to deliver 1.0 and 10.0 ml with 1.0 ml graduations.

Test tube rack.-- Use any of the commercially available test tube racks. If a water bath is to be used for incubation, rubberized or plastic coated racks will be more resistant to corrosion.

Test tubes.-- 150 X 16 mm, screw-top with molded plastic caps (sterile).

Bottles.-- Amber glass, 200 ml capacity.

PROCEDURE

To a sterile test tube, add 1 ml of nonfat milk solution and 10 ml sterile water (boil water for 5 minutes to sterilize and cool to room temperature). To another sterile test tube, add 1 ml of nonfat milk solution and 10 ml of raw sap. Cap and invert tubes to mix contents. Incubate both tubes at 98.6° F (37.5° C) for 30 minutes. After incubation, add 1 ml of resazurin dye to each tube. Invert tubes to mix dye evenly. The control tube color should match the Munsell PB6/10 color swatch and should not change color during the test. (A control color change indicates unsterile nonfat milk, water, or dye solution. A poorly mixed dye also gives an abnormal pinkish color to a control tube.) Except in cases of very severe and active microbial contamination, the sap tube color will match that of the control at this time. Incubate the tubes at 98.6 $\pm$ 1° F (37.5° C) and examine the tubes for a color change to the positive Munsell P6/8 at hourly intervals. The bacterial count in the sap can be estimated from figure 1 showing a graph in which the bacterial counts in raw sap samples were plotted versus the incubation time required for these micro-organisms to reduce the resazurin dye to the Munsell P6/8 end-point color.

DISCUSSION

The figure 1 graph is a curve (the solid line) constructed from 105 determinations and summarizes data submitted to this laboratory from 13 collaborating laboratories. In this curve, the logarithm of the bacterial count in the sap is plotted against the time required for a positive color reaction. The line was fitted by the least squares method. The interrupted lines delineate 95-percent confidence limits.

The maple producer can use this test to predict the approximate quality of sirup produced from a given lot of sap. Table 1 shows the relationship between resazurin (color change) reduction time and sirup quality as observed by workers both at ERRC and in the field. These data indicate that rapid reduction of the dye, 30 minutes or less, identifies a sap of such poor quality that a marketable product could not be made from it. Sap showing a reduction time of 1 hour yields a "black-strap" type of sirup which might be sold as a humectant for tabacco. As the resazurin reduction time lengthens beyond 1 hour, there is a steady upgrading of sirup flavor and color quality until the 4 hour mark is reached. Sap lots showing resazurin reduction times of 4 or more hours yield sirups of the highest quality.

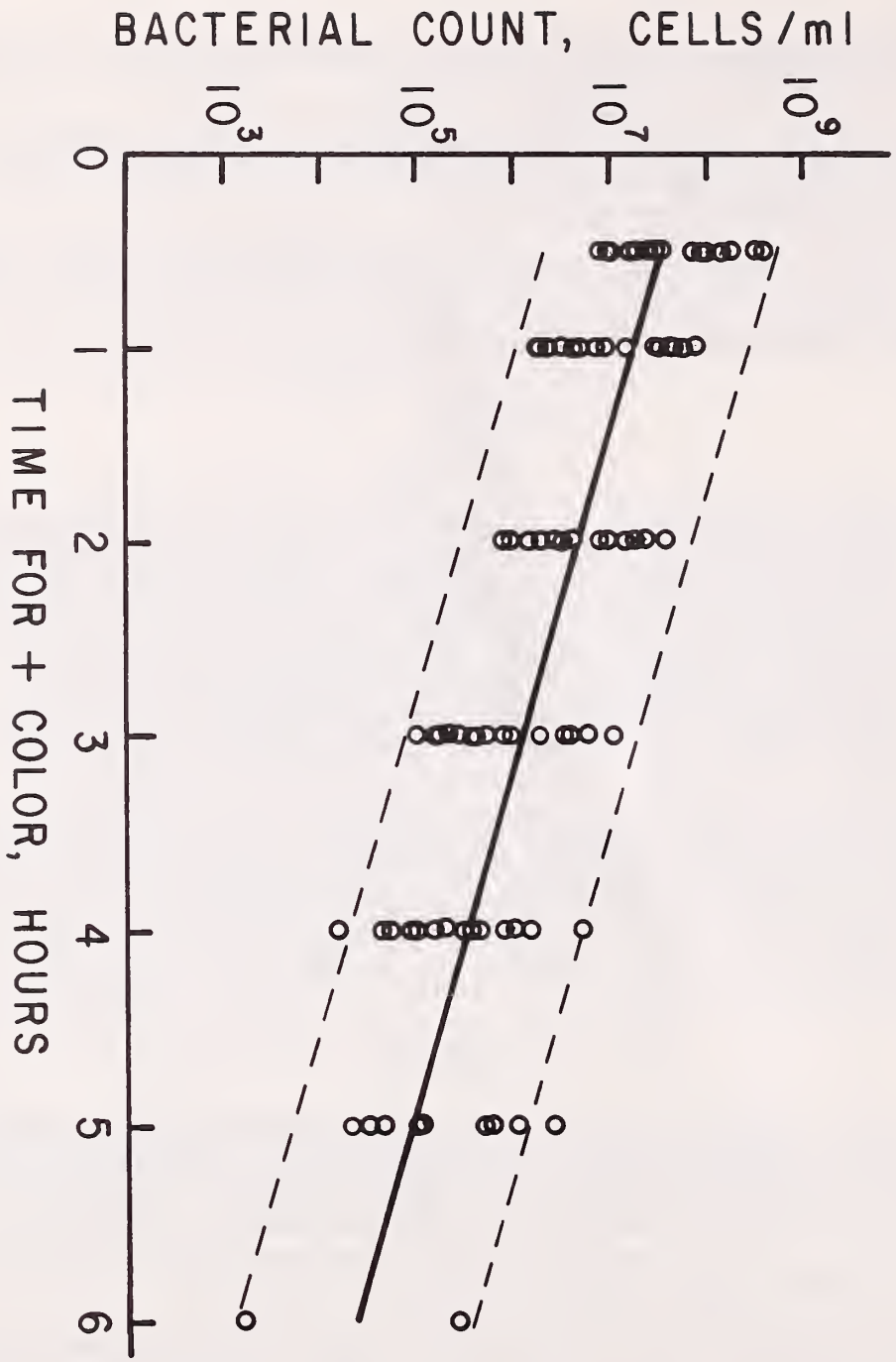

Figure 1.--Relation of bacterial count in raw maple sap to resazurin dye reduction time.

TABLE 1.--Predication of probable quality of maple sirup by the resazurin test of the sap before boiling

Time for resazurin color change	Sirup quality	
	Color	Flavor and odor
Instantaneous	Very light yellow	Putrid.
30 minutes	Very dark amber	Strong "off" flavor.
1 hour	Dark amber	Possible "off".
2 hours	Medium amber	Good maple.
3 hours	Light medium amber	Good maple.
4 hours	Light amber	Delicate maple.

While the maple producer can use this test to predict the quality of the sirup produced from a lot of sap, the main value of this test lies in its ability to quickly detect sap of inferior sanitary quality. By excluding inferior sap from the production line, the maple producer can save the costs of production and the energy which might otherwise be wasted on the manufacture of an unmarketable product.

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